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Short Communication

HPLC analysis of buprenorphine in plasma and urine using coulometric detection

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Introduction

The opioid buprenorphine 21-cyclopropyl-7-[(S)-1-hydroxy-1,2,2 trimethylpropyl]-6, 14-endo-ethanotetrahydrooripavine (**1**) is one of the strongest therapeutically available analgesics today [1, 3]. Buprenorphine is active at very low concentrations and its duration of action is longer than that observed with other opioids. The drug is particularly interesting because of its surprisingly low ability to produce physical dependence. This paper presents a simple and rapid method for a precise and accurate determination of buprenorphine in biological fluids.

Experimental

Chemicals

Analytical grade solvents and reagents were used. Solvents for the mobile phase were HPLC grade. The derivative of buprenorphine, 21-cyclopropyl 7-[2-(3,3-dimethyl-1-butenyl)]-6,14-endo-ethanotetrahydrooripavine (**2**) was used as an internal standard.

Apparatus

A modular liquid chromatograph was used, comprised of a high pressure pump (Constametric III G LDC/Milton Roy, Riviera Beach, Florida, USA), a six-port injector (Model 190 Negretti and Zambra, Southampton, England), a cyanopropylsilane column (Zorbax CN 15 cm × 4.6 mm, 5 μm, Du Pont Instruments, Wilmington, Delaware, USA), a coulometric detector (ESA Coulochem 5100 A, Bedford Massachusetts, USA), a strip chart recorder (Model A 521-1, Houston Instruments, Austin Texas, USA) and an integrator (Model 3392A Hewlett–Packard, Palo Alto, California, USA). A laboratory centrifuge (Dynac2 Clay Adams) and a nitrogen evaporator (Meyer) were used for separating and evaporating the organic layer.

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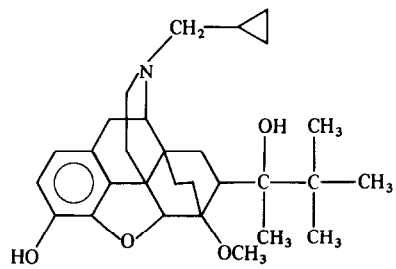

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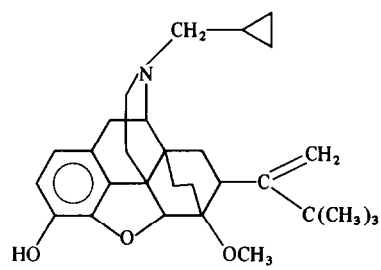

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Chromatographic conditions

Reversed-phase chromatography was performed at room temperature. The optimum mobile phase was found to be a mixture of water–acetonitrile–methanol (60:20:20, v/v/v) containing 0.01 M of the ion pair reagent 1-octane sulphonic acid and 0.005 M tetrabutylammonium chloride. The eluent was adjusted to pH 3.5 with 1 M sodium hydroxide. Tetrabutylammonium chloride was added to improve the peak shape by saturating the unbound silanol groups of the stationary phase. The mobile phase was filtered through a 0.20 μm filter and degassed before use. It was recycled during chromatography. The flow rate was 0.8 ml/min. Electrochemical detection was performed, the first cell (clean-up cell) maintained at +0.18 V and the second cell (detection cell) at +0.38 V. A guard cell maintained at a potential of 0.80 V was placed before the injector to eliminate the electro-active impurities present in the mobile phase. These optimum operating conditions were derived from a hydrodynamic voltammogram (Fig. 1) where the detector response (peak height) is plotted versus the applied potential (*V*). The optimal potential for the detection cell was obtained from the maximum of a signal-to-noise ratio versus applied potential plot (Fig. 2). The potential of the clean-up cell was set at a voltage as close as practicable to the oxidation potential of buprenorphine, in order to obtain a better selectivity.

Extraction procedure

Several different solvents were tried for extracting buprenorphine for plasma and urine. These solvents included hexane, chloroform, toluene and benzene as well as mixtures of chloroform–butanol, chloroform–isopropanol and hexane–ethylacetate.

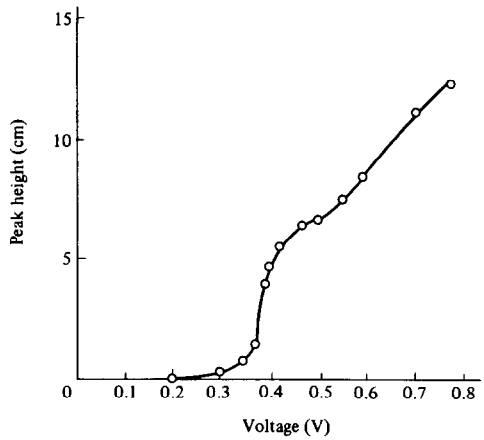

Figure 1
Hydrodynamic voltammogram for buprenorphine. Current–voltage curve determined by application of different oxidation potentials.

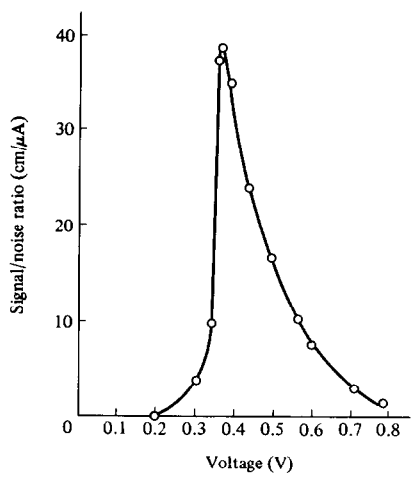

Figure 2
Hydrodynamic voltammogram for buprenorphine. Determination of optimum potential by measuring the signal-to-noise ratio.

Plasma and urine were spiked with drug and internal standard and the pH adjusted to 8.9–9.1 with 0.1 M borate/boric acid buffer pH 9.1. The best results were obtained using the following methods:

Plasma. 1 ml of plasma was spiked with appropriate amounts of drug and internal standard, 1 ml of borate/boric acid buffer (0.1 M), pH 9.1, was added and the pH adjusted to 8.9–9.1. The samples were extracted with 5 ml of benzene by shaking for 30 min. After centrifuging for 20 min at 2000 rpm the organic layer was transferred into fresh tubes and evaporated to dryness under nitrogen. The residue was reconstituted in 100 μl mobile phase. In the low concentration range 80 μl volumes were used for reconstitution.

Urine. Urine was centrifuged and diluted 1:1 or 1:10 before extraction. The diluted urine was first extracted according to the procedure described for plasma. The benzene

layer was then back-extracted into 1 ml 1.0 M HCl, the organic layer discarded and the remaining aqueous layer extracted again into benzene after addition of 1 ml 1.0 M NaOH and 1 ml borate/boric acid buffer (0.1 M).

Blanks for urine and plasma without addition of drug and standard were prepared accordingly.

Results

Calibration curves

Calibration curves were obtained for plasma and urine by plotting the peak height ratio of buprenorphine and the internal standard versus buprenorphine concentrations (Table 1). The curves were linear up to a concentration of 10 μg/ml plasma and 200 μg/ml urine with a correlation coefficient $r > 0.99$. Typical chromatograms after plasma and urine extraction are shown in Fig. 3.

Table 1
Statistics of calibration curves

Medium range	$m \pm s_m$	$b \pm s_b$	s_{xy}	r^2
Plasma 1–50 ng/ml	11.0 ± 0.06	−0.92 ± 0.13	0.325	0.995
Plasma 500–1000 ng/ml	748.0 ± 8.75	−30.5 ± 5.81	10.4	0.995
Plasma 1–10 μg/ml	9.53 ± 0.172	−1.60 ± 0.138	0.155	0.992
Urine 20–400 ng/ml	148.3 ± 1.14	−1.51 ± 0.74	1.460	0.998
Urine 0.2–8 μg/ml	2.67 ± 0.06	−1.11 ± 0.106	0.155	0.992

Buprenorphine concentrations (c) versus peak height ratio (R), $c \pm s_{xy} = (m \pm s_m)R + (b \pm s_b)$.

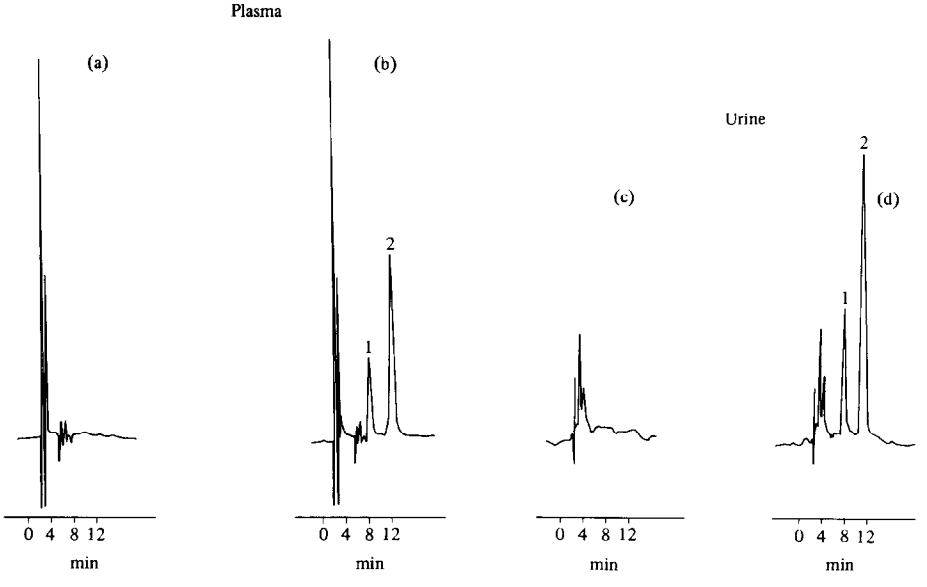

Figure 3
Chromatograms corresponding to extracts of buprenorphine from plasma and urine: (A) blank plasma, (B) plasma spiked with 40 ng/ml buprenorphine (**1**) and internal standard (**2**), (C) blank urine and (D) urine containing 400 ng/ml buprenorphine (**1**).

Sensitivity

The limit of sensitivity for precise quantitation was 1 ng/ml in plasma, however, concentrations of about 500 pg/ml could still be detected. Buprenorphine was determined precisely down to 20 ng/ml urine whereas a concentration of 10 ng/ml was still detectable in urine.

Reproducibility and recovery studies

The precision of the method was studied for plasma and urine by repetitive extraction of samples at different concentrations. Results are shown in Table 2. The average relative standard deviation for the reproducibility study is 3.64% for 10, 50, 150 and 500 ng/ml plasma and 3.12% for 50, 400 and 800 ng/ml urine. The results obtained demonstrate that the method is accurate and precise for quantification of buprenorphine in plasma and urine.

Recovery was studied by comparing the peak height of buprenorphine after extraction from plasma and the peak height obtained after injection of a straight solution at the same concentration. Recovery studies were performed for concentrations of 50, 150 and 500 ng/ml in plasma. The recovery was found to be between 88.4 and 104%. Results are shown in Table 3.

Application

The method was applied to determine buprenorphine levels in dog plasma after a 0.7766 mg/kg i.v. administration of the drug. The results were compared to those obtained with fluorescence detection (E. R. Garrett and V. Ravi Chandran, *Pharmacokinetics of Buprenorphine*, in preparation).

Table 2
Accuracy and precision of the assay

Medium	Buprenorphine conc. (ng/ml)	*n*	Assayed mean (ng/ml)	Error (%)	CV (%)
Plasma	10	5	9.93	0.70	4.36
Plasma	50	5	51.31	2.62	7.05
Plasma	150	5	147.50	1.67	2.08
Plasma	500	5	502.68	0.54	1.08
Urine	50	4	49.40	1.20	5.05
Urine	400	5	407.21	1.80	2.17
Urine	800	4	784.53	1.93	2.15

Table 3
Recovery study in plasma

Buprenorphine conc. (ng/ml)	*n*	Mean recovery (%)	SD
50	5	104	9.2
150	5	90.4	8.3
500	5	88.4	2.15

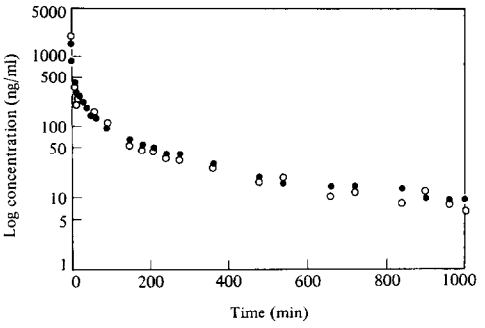

Figure 4
Plasma concentrations of buprenorphine after i.v. administration of the drug in dog. ●, fluorimetric detection; ○ coulometric detection.

Semilogarithmic plots of the concentrations (as base, in ng/ml plasma) versus time (min) are shown in Fig. 4. The close agreement between the two curves demonstrates the applicability of both methods of detection.

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